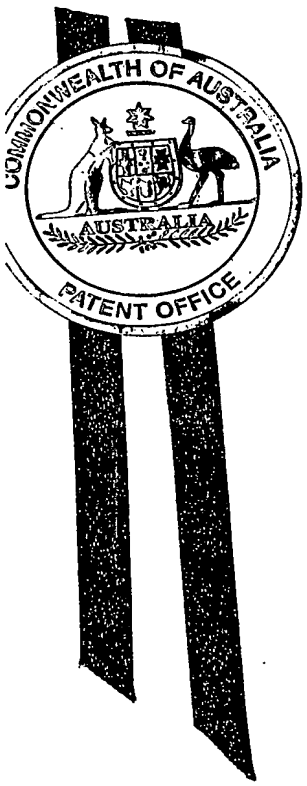
PCT/AU04/001772

Australian Government

Patent Office
Canberra

I, LEANNE MYNOTT, MANAGER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2003906981 for a patent by MONASH UNIVERSITY as filed on 16 December 2003.



WITNESS my hand this
Eleventh day of January 2005

LEANNE MYNOTT
MANAGER EXAMINATION SUPPORT
AND SALES

Regulation 3.2

Monash University

AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"Methods of inducing analgesia"

The invention is described in the following statement:

METHODS OF INDUCING ANALGESIA

FIELD OF THE INVENTION

The present invention relates generally to methods of inducing analgesia in response to inflammatory or neuropathic pain, which involve administration of flupirtine or its derivatives, optionally in association with one or more other analgesic agents such as opioid compounds. The present invention also relates to compositions and kits useful in inducing analgesia in response to neuropathic or inflammatory pain.

BACKGROUND OF THE INVENTION

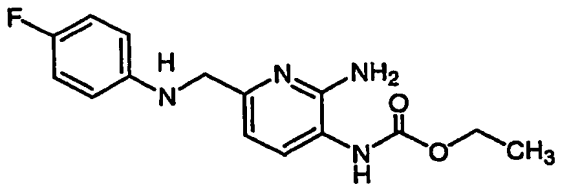
The present invention relates generally to the induction of analgesia in response to neuropathic or inflammatory pain. In considering the approaches to treatment of pain it is important to understand the distinction between acute and chronic pain. Acute pain occurs as a result of tissue injury or inflammation, and is mediated by chemical, mechanical or thermal stimulation of pain receptors. In this context "inflammatory pain" is considered to constitute a subset of acute pain or chronic pain that results from inflammatory processes, such as may arise in the case of arthritis or infections and neoplasia or tumour related hypertrophy. Tumour or cancer associated pain is therefore considered to fall within the category of inflammatory pain. In contrast to acute pain, chronic pain in itself constitutes a disease, which serves no protective biological function. Chronic pain is unrelenting and can persist for years after an initial injury. Chronic, non-malignant pain predominantly constitutes chronic inflammatory pain (eg arthritis) or "neuropathic pain", which can be defined as pain initiated or caused by a primary lesion or dysfunction within the nervous system[1]. Neuropathic pain is associated with a variety of disease states and presents in the clinic with a range of symptoms[2].

Neuropathic pain is often reported as having a lancinating or continuous burning character and is often associated with the appearance of abnormal sensory signs such as allodynia and hyperalgesia. Allodynia is defined as pain resulting from a stimulus that does not

normally elicit a painful response, and hyperalgesia is characterised by an increased pain response to a normally painful stimulus. Some disorders characterised by neuropathic pain include monoradiculopathies, trigeminal neuralgia, postherpetic neuralgia, phantom limb pain, complex regional pain syndromes and the various peripheral neuropathies. Neuropathic pain may also be associated with, with diabetes, with radio- or chemotherapy, with infections such as HIV and AIDS. Neuropathic pain may also result as a side effect of drug treatment or abuse.

Although there are numerous available therapies for acute pains caused by inflammatory processes or acute injury, especially including treatment with opioid and non-steroidal anti-inflammatory drugs (NSAIDs), neuropathic pain is an area of largely unmet therapeutic need. Due to the distinct pathophysiological mechanisms associated with neuropathic pain relative to inflammatory pains, agents useful in treatment of inflammatory and other pains generally have reduced effectiveness in neuropathic pain treatment. In particular, the effectiveness of opioids in treatment of neuropathic pain is diminished relative to inflammatory pain treatment, and the dose response curve of opioids in neuropathic pain is shifted to the right of that for inflammatory pain[5]. Frequent and sustained use of opioids is associated with addiction and the development of tolerance. Furthermore, the side effects of the opioids including euphoric effect, emetic effect, spastic constipation, increased smooth muscle tone and sedation reduce the therapeutic effectiveness of the opioids and are dose limiting.

The conventional pharmacological mainstays of clinical management of neuropathic pain are the tricyclic anti-depressants and certain anti-convulsants[3,4], but even these achieve clinically significant pain relief (that is greater than 50% pain relief) in less than 50% of patients. These agents are also associated with significant side effect profiles.



Formula I

Flupirtine (Formula I) ([2-amino-6-[[(4-fluorophenyl)methyl]amino]-3-pyridinyl]carbamic acid ethyl ester), which is commonly prepared in the form of the hydrochloride or maleate, is disclosed in German Pat. No. 1,795,858 (hydrochloride) and US patent no. 4,481,205 (maleate), the contents of which are included herein in their entirety by way of reference. Flupirtine is an analgesic agent that exhibits some muscle-relaxing activity and its action is mediated by NMDA receptor antagonism and α_2-adrenoceptor agonism. Flupirtine has been reported to have no dependence potential[6] and to have no affinity for opiate receptors[7].

Although flupirtine is an analgesic in clinical use in Germany, it is not in widespread clinical use elsewhere. The probable reason for this is that its analgesic efficacy is limited by dose limiting side effects, including dizziness, nausea, sleep disturbance, headache, dry mouth, pruritis and gastrointestinal complaints. A side effect of particular concern, which limits administration dosages, is the induction of sedation.

US patent no. 5,521,178 to Asta Medica AG ("the Asta patent") discloses the combined use of Flupirtine and morphine for the treatment of pain and the prevention of morphine dependence. The Asta patent discusses the following adverse side effects associated with morphine administration: euphoric effect, emetic effect, spastic constipation and increase in smooth muscle tone. The Asta patent also makes reference to the general aim in adopting combination analgesics of reducing the amount of analgesic compounds administered or enhancing their inadequate analgesic effect. There is further disclosure that administration of flupirtine by itself produces an antinociceptive effect of 45%, whereas the administration of the combination with morphine yields an effect of 100%.

There is, however, only limited experimental data provided within the Asta patent, and the document provides no disclosure or suggestion that the combination of flupirtine and morphine could reduce morphine's sedative effect or could be effective in treatment of neuropathic pain. The present inventors have determined that adoption of combined administration of Flupirtine and other analgesic agents, such as opioids, is effective in treatment of neuropathic and inflammatory pain and that in such combination therapies the sedative effects of the analgesic agents can be minimised while maintaining an effective analgesic dose. The inventors have also devised therapies involving administration of Flupirtine alone, which do not give rise to overt sedation and are effective for treatment of neuropathic and inflammatory pain.

There is a pressing need for improved regimes for treatment of neuropathic pain and to provide effective alternative treatments for inflammatory pain. It is in view of this background that the present invention has been conceived. Other aspects of the present invention will become apparent from the following detailed description thereof.

SUMMARY OF THE INVENTION

According to one embodiment of the present invention there is provided a method of inducing analgesia in response to inflammatory or neuropathic pain in a mammal, comprising administering to the mammal an effective amount of flupirtine or a pharmaceutically acceptable derivative thereof. In a preferred embodiment of the invention the pain is neuropathic pain. Preferably the method does not induce overt sedation.

According to another embodiment of the present invention there is provided a method of inducing analgesia in response to inflammatory or neuropathic pain in a mammal without inducing overt sedation, comprising administering to the mammal an effective amount of flupirtine or a pharmaceutically acceptable derivative thereof. In a preferred embodiment of the invention the pain is neuropathic pain.

In a still further embodiment of the present invention there is provided a method of inducing analgesia in response to inflammatory or neuropathic pain in a mammal, comprising concurrently, separately or sequentially administering to the mammal effective amounts of an analgesic agent and flupirtine or a pharmaceutically acceptable derivative thereof. Preferably the analgesic agent and flupirtine or a pharmaceutically acceptable derivative thereof are administered in synergistically effective amounts. Preferably the method does not induce overt sedation. Preferably the analgesic agent is an opioid. Preferably the opioid is selected from one or more of fentanyl, oxycodone, codeine, dihydrocodeine, dihydrocodeinone enol acetate, morphine, desomorphine, apomorphine, diamorphine, pethidine, methadone, dextropropoxyphene, pentazocine, dextromoramide, oxymorphone, hydromorphone, dihydromorphine, noscapine, papaverine, papaveretum, alfentanil, buprenorphine and tramadol and pharmaceutically acceptable derivatives thereof.

In a still further embodiment of the present invention there is provided a method of inducing analgesia in response to inflammatory or neuropathic pain in a mammal without inducing overt sedation, comprising concurrently, separately or sequentially administering to the mammal effective amounts of an analgesic agent and flupirtine or a pharmaceutically acceptable derivative thereof. Preferably the analgesic agent and flupirtine or a pharmaceutically acceptable derivative thereof are administered in synergistically effective amounts. Preferably the analgesic agent is an opioid. Preferably the opioid is selected from one or more of the opioids listed above or pharmaceutically acceptable derivatives thereof.

In another embodiment the invention relates to the use of flupirtine or a pharmaceutically acceptable derivative thereof in the manufacture of a medicament for inducing analgesia in response to inflammatory or neuropathic pain. Preferably the analgesia is induced without overt sedation and preferably the pain is neuropathic pain.

In a further embodiment the invention relates to the use of an analgesic agent and flupirtine or a pharmaceutically acceptable derivative thereof, in the manufacture of a medicament for inducing analgesia in response to inflammatory or neuropathic pain. Preferably the analgesia is induced without overt sedation and preferably the pain is neuropathic pain. In a preferred embodiment the analgesic agent is an opioid and preferably the opioid is selected from one or more of the opioids listed above or pharmaceutically acceptable derivatives thereof.

In a still further embodiment of the present invention there is provided a kit for inducing analgesia in response to inflammatory or neuropathic pain in a mammal comprising an analgesic agent and flupirtine or a pharmaceutically acceptable derivative thereof. In a preferred embodiment the analgesic agent is an opioid and preferably the opioid is selected from one or more of the opioids listed above or pharmaceutically acceptable derivatives thereof.

The compounds according to the invention may be administered, *inter alia,* orally, transmucosally, rectally, subcutaneously, intravenously, intramuscularly, intraperitoneally, intragastrically, intrathecally, transdermally or intestinally. In particularly preferred forms of the invention, the compounds are administered orally or transdermally.

Preferably the flupirtine or pharmaceutically acceptable derivative thereof is administered at a dose of between about 0.5 mg/kg and about 20 mg/kg, at intervals of between about 3 hours and about 24 hours, when administered either alone or in combination with analgesic agent. Preferably the intervals are between about 4 hours and about 8 hours. Most preferably the administration is at intervals of about 6 hours.

In a particularly preferred embodiment of the invention the mammal is a human.

BRIEF DESCRIPTION OF THE FIGURES

The present invention will be further described with reference to the following figures, wherein:

Fig. 1 shows time response curves for carrageenan-induced hyperalgesia in male Wistar rats, where paw flick latency (seconds) is plotted against time (minutes) for saline controls (diamonds), flupirtine at 5 mg/kg (squares), flupirtine at 10 mg/kg (stars), morphine at 0.8 mg/kg (vertical bars), morphine at 1.6 mg/kg (horizontal bars), the combination of flupirtine at 5 mg/kg with morphine at 0.4 mg/kg (squares) and the combination of flupirtine at 10 mg/kg with morphine at 0.4 mg/kg (circles).

Fig. 2 shows time response curves for antinociception assessed with the Electrical Current Threshold (ECT) test in male Wistar rats, where standardised ECT value as a ratio against the control is plotted against time for saline controls (triangles), flupirtine at 5 mg/kg (diamonds), morphine at 0.4 mg/kg (circles) and the combination of flupirtine at 5 mg/kg with morphine at 0.4 mg/kg (squares);

Fig. 3 shows antinociceptive effects in streptozotocin-induced diabetic neuropathy in male Wistar rats, where paw withdrawal threshold (grams) is plotted against time (minutes), where zero time is time of test drug injection, for saline controls (diamonds), flupirtine at 5 mg/kg (squares), flupirtine at 10 mg/kg (triangles), morphine at 1.6 mg/kg (crosses), morphine at 3.2 mg/kg (stars), the combination of flupirtine at 5 mg/kg with morphine at 3.2 mg/kg (closed circles) and the combination of flupirtine at 10 mg/kg with morphine at 1.6 mg/kg (open squares), with results for weight matched non-diabetic controls shown with an open circle.

DETAILED DESCRIPTION OF THE INVENTION

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

As conveyed above, the present invention relates to methods of inducing analgesia in response to neuropathic pain in a mammal. In this context the term "mammal" is intended to encompass both humans and other mammals such as laboratory animals including rats, mice, simians and guinea pigs, domestic animals including cats, dogs, rabbits, agricultural animals including cattle, sheep, goats, horses and pigs and captive wild animals such as lions, tigers, elephants and the like.

Throughout this specification, the term "neuropathic pain" is to be understood to mean pain initiated or caused by a primary lesion or dysfunction within the nervous system. Examples of categories of neuropathic pain that may be treated by the methods of the invention include monoradiculopathies, trigeminal neuralgia, postherpetic neuralgia, phantom limb pain, complex regional pain syndromes, neuropathic pain associated with AIDS and infection with the human immunodeficiency virus and the various peripheral neuropathies, including, but not limited to drug-induced and diabetic neuropathies.

The term "inflammatory pain" is intended to describe the subset of acute and chronic pain that results from inflammatory processes, such as may arise in the case of infections, arthritis and neoplasia or tumour related hypertrophy. Tumour or cancer associated pain is therefore considered to fall within the category of inflammatory pain.

It is the intention of the methods according to the present invention to induce analgesia in response to inflammatory and/or neuropathic pain being suffered by a mammalian, preferably human, patient. In this context the term "analgesia" is intended to describe a state of reduced sensibility to pain, which preferably occurs without overt sedation and

preferably without an effect upon the sense of touch. Preferably, the sensibility to pain is reduced by at least 30%, preferably at least 50%, more preferably at least 70% and particularly preferably at least 85%. In the most preferred aspect of the invention the sensibility to the neuropathic pain is completely, or substantially completely, removed. To assess the level of reduction of sensibility to pain associated with the analgesia induced by the methods according to the present invention it is possible to conduct tests such as the short form McGill pain questionnaire and/or visual analogue scale for pain intensity and/or verbal rating scale for pain intensity and/or measurement of tactile allodynia using von Frey hairs or similar device. These tests are standard tests within the art and would be well known to the skilled person.

By the term "overt sedation" it is intended to convey that the methods (and compositions) of the invention do not result in practically meaningful sedation of the patent, ie significant, visible or apparent drowsiness or unconsciousness of the patient being treated. Thus, the treatment methods of the invention do not result in sleepiness or drowsiness in the patient that interfere with, or inhibit, the activities associated with day to day living, such as driving a motor vehicle or operating machinery for human subjects, or feeding and grooming for animal subjects.

In another aspect of the present invention the method of inducing analgesia in response to inflammatory or neuropathic pain may involve concurrent, separate or sequential administration to the mammal in need of such treatment of additively, or more preferably, synergistically effective amounts of flupirtine, or a pharmaceutically acceptable derivative thereof, and another analgesic agent such as an opioid. Collectively the flupirtine or pharmaceutically acceptable derivative thereof and the other analgesic agent will be referred to as the "active agents". A synergistically effective amount of flupirtine or a pharmaceutically acceptable derivative thereof, when administered concurrently, separately or sequentially with an opioid may restore opioid responsiveness to inflammatory or neuropathic pain. The active agents may be administered either as a combined form, ie a single composition containing the active agents, or as discrete dosages. The active agents will preferably be administered within a time frame allowing

the desired additive or synergistic analgesic effect to be achieved. That is, the timing of administration should allow each of the active agents or their active metabolites to simultaneously be present within the patient within their respective therapeutic concentration ranges.

The term "analgesic agent" is intended to encompass known and as yet unknown compounds (including pharmaceutically acceptable derivatives thereof) that are effective for treatment of pain in mammals, including: aspirin; paracetamol; NSAIDs such as ibuprofen, indomethacin and phenylbutazone; the opioids; tricyclic antidepressants such as amitryptyline{6626}; anticonvulsants such as carbamazepine and sodium valproate; local anaesthetics such as lignocaine, mexiletine; NMDA antagonists such as dextromethorphan or ketamine; neurosteroid analgesics such as alphadolone; and GABApentin. The term is intended to particularly encompass analgesics in relation to which dose limiting side effects are associated, and especially those associated with induction of sedation. Particularly preferred other analgesic agents are the opioids.

As used herein, opioid compounds (opioids) include any compound that is physiologically acceptable in mammalian systems and is a full or at least partial agonist of an opioid receptor. Opioid compounds are well known and include naturally occurring compounds derived from opium such as codeine, morphine and papavarine as well as derivatives of such compounds that generally have structural similarity as well as other structurally unrelated compounds that agonise an opioid receptor present in a mammalian system. Specific examples of opioid compounds contemplated by the present invention include: fentanyl, oxycodone, codeine, dihydrocodeine, dihydrocodeinone enol acetate, morphine, desomorphine, apomorphine, diamorphine, pethidine, methadone, dextropropoxyphene, pentazocine, dextromoramide, oxymorphone, hydromorphone, dihydromorphine, noscapine, papaverine, papaveretum, alfentanil, buprenorphine and tramadol and pharmaceutically acceptable derivatives thereof.

The phrase "pharmaceutically acceptable derivative" is intended to convey any pharmaceutically acceptable tautomer, salt, pro-drug, hydrate, solvate, metabolite or other

compound which, upon administration to the subject, is capable of providing (directly or indirectly) the compound concerned or a physiologically (eg analgesically) active compound, metabolite or residue thereof. An example of a suitable derivative is an ester formed from reaction of an OH or SH group with a suitable carboxylic acid, for example C_{1-3}alkyl-CO_2H, and HO_2C-$(CH_2)_n$-CO_2H (where n is 1-10, preferably 1-4), and CO_2H-CH_2phenyl.

Thus, the active compounds may be in crystalline form, either as the free compounds or as solvates (eg hydrates). Methods of solvation are generally known within the art.

The salts of the active compounds of the invention are preferably pharmaceutically acceptable, but it will be appreciated that non-pharmaceutically acceptable salts also fall within the scope of the present invention, since these are useful as intermediates in the preparation of pharmaceutically acceptable salts. Examples of pharmaceutically acceptable salts include salts of pharmaceutically acceptable cations such as sodium, potassium, lithium, calcium, magnesium, ammonium and alkylammonium; acid addition salts of pharmaceutically acceptable inorganic acids such as hydrochloric, orthophosphoric, sulfuric, phosphoric, nitric, carbonic, boric, sulfamic and hydrobromic acids; or salts of pharmaceutically acceptable organic acids such as acetic, propionic, butyric, tartaric, maleic, hydroxymaleic, fumaric, citric, lactic, mucic, gluconic, benzoic, succinic, oxalic, phenylacetic, methanesulphonic, trihalomethanesulfphonic, toluenesulphonic, benzenesulphonic, salicyclic, sulphanilic, aspartic, glutamic, edetic, stearic, palmitic, oleic, lauric, pantothenic, tannic, ascorbic and valeric acids.

The term "pro-drug" is used herein in its broadest sense to include those compounds which can be converted *in vivo* to the compound of interest (eg by enzymatic or hydrolytic cleavage). Examples thereof include esters, such as acetates of hydroxy or thio groups, as well as phosphates and sulphonates. Processes for acylating hydroxy or thio groups are known in the art, eg by reacting an alcohol (hydroxy group), or thio group, with a carboxylic acid. Other examples of suitable pro-drugs are described in *Design of Prodrugs*, H. Bundgaard, Elsevier, 1985, the disclosure of which is included herein in its

entirety by way of reference.

The term "metabolite" includes any compound into which the active agents can be converted *in vivo* once administered to the subject. Examples of such metabolites are glucuronides, sulphates and hydroxylates.

It will be understood that active agents as described herein may exist in tautomeric forms. The term "tautomer" is used herein in its broadest sense to include compounds capable of existing in a state of equilibrium between two isomeric forms. Such compounds may differ in the bond connecting two atoms or groups and the position of these atoms or groups in the compound. A specific example is keto-enol tautomerism.

The compounds of the invention may be electrically neutral or may take the form of polycations, having associated anions for electrical neutrality. Suitable associated anions include sulfate, tartrate, citrate, chloride, nitrate, nitrite, phosphate, perchlorate, halosulfonate or trihalomethylsulfonate.

The active agents may be administered for therapy by any suitable route. It will be understood that the active agents are preferably administered via a route that does not result in overt sedation of the subject. Suitable routes of administration may include oral, rectal, nasal, inhalation of aerosols or particulates, topical (including buccal and sublingual), transdermal, vaginal, intravesical and parenteral (including subcutaneous, intramuscular, intravenous, intrasternal, intrathecal, epidural and intradermal). Preferably, administration of the active agents will be by a route resulting in first presentation of the compound to the stomach of the subject. In a particularly preferred embodiment of the invention, the active agents are administered via an oral route. In another preferred embodiment the active agents are administered by the transdermal route. However it will be appreciated that the preferred route will vary with the condition and age of the subject, the nature of the inflammatory or neuropathic pain being treated, its location within the subject and the judgement of the physician or veterinarian. It will also be understood that individual active agents may be administered by distinct routes

As used herein, an "effective amount" refers to an amount of active agent that provides the desired analgesic activity when administered according to a suitable dosing regime. Preferably the amount active agent is an amount that provides the desired analgesic activity without causing overt sedation. Dosing may occur at intervals of minutes, hours, days, weeks or months. Suitable dosage amounts and regimes can be determined by the attending physician or veterinarian. For example, flupirtine or pharmaceutically acceptable derivatives thereof, may be administered to a subject at a rate of between about 0.5 to about 20 mg/kg every six hours. Dosing of the analgesic agent, such as an opioid, can be determined by the attending physician in accordance with dosing rates in practice. For example, fentanyl can be administered in an amount of about 100 μg whereas morphine may be administered in an amount of 10 mg, also on an hourly basis. Naturally the administration amounts may be varied if administration is conducted more or less frequently, such as by continuous infusion, by regular dose every few minutes or by administration every 10, 20, 30 or 40 minutes or every 1, 2, 3, 4, 6, 8, 10, 12, 16 or 24 hours, for example. In many instances administration will be conducted simply on the basis of when the patient requires pain relief.

The present invention also relates to compositions comprising flupirtine or a pharmaceutically acceptable derivative thereof, optionally with another analgesic agent such as an opioid, together with one or more pharmaceutically acceptable additives and optionally other medicaments. The pharmaceutically acceptable additives may be in the form of carriers, diluents, adjuvants and/or excipients and they include all conventional solvents, dispersion agents, fillers, solid carriers, coating agents, antifungal or antibacterial agents, dermal penetration agents, surfactants, isotonic and absorption agents and slow or controlled release matrices. The active agents may be presented in the form of a kit of components adapted for allowing concurrent, separate or sequential administration of the active agents. Each carrier, diluent, adjuvant and/or excipient must be "pharmaceutically acceptable" in the sense of being compatible with the other ingredients of the composition and physiologically tolerated by the subject. The compositions may conveniently be presented in unit dosage form and may be prepared by methods well known in the art of

pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier, which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers, diluents, adjuvants and/or excipients or finely divided solid carriers or both, and then if necessary shaping the product.

Compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, sachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous phase or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. inert diluent, preservative disintegrant (eg. sodium starch glycollate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Moulded tablets may be made my moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Compositions suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the composition isotonic with the blood of the intended subject; and aqueous and non-aqueous sterile suspensions which may include suspended agents and thickening agents. The compositions may be presented in a unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried

(lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Compositions suitable for topical administration to the skin, ie transdermal administration, may comprise the active agents dissolved or suspended in any suitable carrier or base and may be in the form of lotions, gels, creams, pastes, ointments and the like. Suitable carriers may include mineral oil, propylene glycol, waxes, polyoxyethylene and long chain alcohols. Transdermal devices, such as patches may also be used and may comprise a microporous membrane made from suitable material such as cellulose nitrate/acetate, propylene and polycarbonates. The patches may also contain suitable skin adhesive and backing materials.

The compounds of formula I may also be presented as implants, which may comprise a drug bearing polymeric device wherein the polymer is biocompatible and non-toxic. Suitable polymers may include hydrogels, silicones, polyethylenes and biodegradable polymers.

The compounds of the invention may be administered in a sustained (ie controlled) or slow release form. A sustained release preparation is one in which the active ingredient is slowly released within the body of the subject once administered and maintains the desired drug concentration over a minimum period of time. The preparation of sustained release formulations is well understood by persons skilled in the art. Dosage forms may include oral forms, implants and transdermal forms. For slow release administration, the active ingredients may be suspended as slow release particles or within liposomes, for example.

It should be understood that in addition to the ingredients particularly mentioned above, the compositions of this invention may include other agents conventional in the art, having regard to the type of composition in question. For example, agents suitable for oral administration may include such further agents as binders, sweetners, thickeners,

flavouring agents, disintegrating agents, coating agents, preservatives, lubricants and/or time delay agents.

Other details of pharmaceutically acceptable carriers, diluents and excipients and methods of preparing pharmaceutical compositions and formulations are provided in Remmingtons Pharmaceutical Sciences 18th Edition, 1990, Mack Publishing Co., Easton, Pennsylvania, USA, the disclosure of which is included herein in its entirety by way of reference.

The active agents for use in the invention may also be presented for use in veterinary compositions. These may be prepared by any suitable means known in the art. Examples of such compositions include those adapted for:

(a) oral administration, eg drenches including aqueous and non-aqueous solutions or suspensions, tablets, boluses, powders, granules, pellets for admixture with feedstuffs, pastes for application to the tongue;

(b) parenteral administration, eg subcutaneous, intramuscular or intravenous injection as a sterile solution or suspension;

(c) topical application, eg creams, ointments, gels, lotions, etc.

In a particularly preferred embodiment of the invention the active agents are administered orally, preferably in the form of a tablet, capsule, lozenge or liquid. The administered composition will preferably include a surfactant and/or solubility improver. A suitable solubility improver is water-soluble polyethoxylated caster oil and an example of a suitable surfactant is Cremophor EL. Dose ranges suitable for flupirtine or pharmaceutical derivatives thereof are for example 100 to 1500 mg orally, every six hours. Suitable dose ranges for morphine are 2.5 to 20 mg every 3 to 6 hours and for oxycodone and other opioids 2 to 50 mg every 3 to 12 hours, for example.

The present invention will now be further described with reference to the following examples, which are intended for the purpose of illustration only and are not intended to limit the generality hereinbefore described.

EXAMPLES

The question addressed in the experiments reported in the examples is whether flupirtine can lead to greater antinociceptive and analgesic effects when combined with morphine, but at lower doses such that side effects of both drugs (eg sedation) can be avoided.

The sedative effects of both drugs were studied using the rotarod test (example 1). This test assesses the ability of rats to walk on a rotating drum. Doses of drugs and combinations of those drugs that cause no decrement in this ability were in this manner identified. The identified non-sedative doses of drugs used singly and in combination were then tested for antinociceptive effects in models of pain, where the following nociceptive paradigms were adopted:

(a) the electrical current threshold test (example 2);

(b) carrageenan-induced paw inflammation (example 3); and

(c) streptozotocin-induced diabetic neuropathy (example 4).

All experiments reported in the examples were performed on male Wistar rats (wt 150-200g for examples 1 to 3) and (wt 65 - 80g for example 4) in an observer-blinded fashion with parallel saline vehicle controls and all drug solutions and vehicle were given intraperitoneally (ip) in a volume of 1.0ml.

Example 1

Rotarod Test

The rats were naïve to the drugs with no previous exposure to the rotarod test. They were placed on the rotarod accelerator treadmill (7650 accelerator rotarod, Ugo Basile, Italy) set at the minimum speed for two training sessions of 1-2 minutes separated by an interval of 30-60 minutes. After this conditioning period the ip injection of vehicle, drug, or drug combination was given. Five minutes later the animals were placed onto the rotarod at a constant speed of 4 revolutions per minute. As the animal took grip of the drum the accelerator mode was selected on the treadmill, i.e. the rotation rate of the drum was

increased linearly at the rate of 20 revolutions per minute every minute thereafter. The time was measured from the start of the acceleration period until the rat fell off the drum; this was the control (pre-treatment) performance time for each rat. A cut-off or maximum run time for the test was 2 minutes because normal non-sedated rats all ran for 2 minutes at which time the test was terminated. This test was performed on each rat for 30 minutes at intervals of 10 minutes between each run. The shortest run time measured after drug injection was identified during the 30-minute test period for each rat. These values were combined for each drug at each dose to calculate means ± SEM. The data from saline treated vehicle controls were compared with the data following drug injections using one-way ANOVA with Tukey Kramer post hoc test. These comparisons allowed definition of drug doses that caused sedation.

Groups of rats were tested with the rotarod as above with the following treatments:

(a) Saline

(b) Morphine at doses of 0.4, 0.8, 1.6, 3.2, and 6.4 mg/kg

(c) Flupirtine at doses of 5, 10 and 20 mg/kg

(d) A combination of flupirtine at 5mg/kg with morphine at 0.4 mg/kg

(e) A combination of flupirtine at 10 mg/kg with morphine at 1.6 mg/kg

Table 1 shows the results of those experiments.

Table 1

treatment	lowest run time (s)		
	n	mean	SD
saline control	30	119.2	2.8
flupirtine 5 mg/kg ip alone	18	118.4	6.1
flupirtine 10 mg/kg ip alone	20	107.7	36.7
flupirtine 20 mg/kg ip alone*	10	58.1*	54.5
morphine 0.4 mg/kg ip alone	10	120	0
morphine 0.8 mg/kg ip alone	10	120	0
morphine 1.6 mg/kg ip alone	10	110.4	19
morphine 3.2 mg/kg ip alone	10	99.6	41.7
morphine 6.4 mg/kg ip alone*	10	60*	41.7
flupirtine 5.0 mg/kg + morphine 0.4 mg/kg together ip	10	119.5	1.3
flupirtine 10 mg/kg + morphine 1.6 mg/kg together ip	10	117	4.45

one way Anova + Tukey-Kramer post-hoc test: compared with saline control *$p < 0.05$

It can be concluded from these experiments that sedation is caused by doses of flupirtine greater than 10 mg/kg and morphine greater than 3.2 mg/kg.

Example 2

Carrageenan Paw Inflammation and Paw Flick Test of Nociception

Experimental inflammation of the right hind paw was induced by an intraplantar injection of carrageenan (Sigma-Aldrich Pty. Ltd. Australia; 100μl of a 2% carrageenan solution in saline). Time was allowed for the induction of inflammation. Paw withdrawal latencies were measured using an infrared beam focussed onto the plantar surface of the right hind paw in freely moving animals using apparatus from Ugo Basile.

Paw withdrawal latencies were measured before the induction of inflammation with carrageenan injections until 3 stable readings were obtained (-20, -10 and 0, as shown in table 2 and figure 1). Once inflammation was established, paw thresholds were measured 60, 110 and 120 minutes after the carrageenan injection to confirm the development of hyperalgesia; a decrease in paw withdrawal latency typically from control pre-carrageenan level of 12 seconds down to 6 seconds. A test drug or drug combination was injected and paw pressure values were measured at 10-minute intervals for the following 40 minutes. Replicate values of paw withdrawal times for time of measurement and drug treatment were combined to calculate mean ± SEM.

The following drug treatments were given to separate groups of rats:

- Saline controls
- Flupirtine at doses of 5 and 10 mg/kg alone
- Morphine at doses of 0.4, 0.8 and 1.6 mg/kg alone
- Combinations of flupirtine at 5 and 10 mg/kg with morphine at 0.4 mg/kg

Time response curves were plotted to determine peak drug effect as shown in figure 1.

It can be seen that the effect of the ip drug injection reaches a plateau from 140 to 160 minutes. The values for all withdrawal latencies in each group were combined for testing times -20, -10 and 0 (pre-treatment) and also for 140, 150 and 160 minute readings (post-treatment). The values are shown in the table 2.

Table 2

Summary of results with carrageenan-induced paw inflammation

	pre-treatment			post-treatment		
TREATMENT	mean	SD	n	mean	SD	n
saline controls	10.98	2.27	72	6.22	2.18	72
flupirtine 5 mg/kg ip alone	10.90	2.80	30	5.82	1.70	30
flupirtine 10 mg/kg alone	10.97	2.42	24	5.51	2.13	24
morphine 0.4 mg/kg ip alone	12.10	2.30	36	5.76	3.10	36
morphine 0.8 mg/kg alone	10.02	1.75	27	4.88	1.67	27
morphine 1.6 mg/kg alone	10.30	2.48	72	8.88	3.15	72
flupirtine 5 mg/kg and morphine 0.4 mg/kg ip together	11.60	2.25	72	8.75	3.31	72
flupirtine 10 mg/kg and morphine 0.4 mg/kg ip together	9.66	1.46	54	10.34	4.02	54

Flupirtine 5 and 10 mg/kg or morphine 0.4 and 0.8 mg/kg alone had no effect on carrageenan-induced hyperalgesia. The combination of flupirtine 5 mg/kg with morphine 0.4mg/kg caused significant reversal of carrageenan-induced hyperalgesia and this was equal to the effect of 1.6mg/kg morphine given alone; flupirtine increased the antinociceptive effect of morphine fourfold. Flupirtine 5mg/kg in combination with morphine 0.4 mg/kg led to significantly less hyperalgesia compared with saline or either drug alone *$p<0.001$ one way ANOVA with Tukey-Kramer post hoc test. Finally, complete reversal of carrageenan-induced hyperalgesia was caused by 10mg/kg flupirtine in combination with 0.4mg/kg morphine i.e., doses of two drugs that were ineffective when given alone caused complete antinociception in this model of neuropathic pain ($p>0.05$ in comparison with pre carrageenan levels (at -20, -10 and 0 mins in graph above) - one way ANOVA with Tukey-Kramer post hoc test). None of these doses or combinations of drugs caused sedation in the rotarod test.

Example 3

Electrical Current Threshold Test

Rats were placed in a restrainer and two surface electrodes were placed on the tail, 2 and 5 cm from the base. Electrical current (50Hz, 2ms pulses, 0-10mA) was passed through these electrodes to determine the minimum current necessary that caused the rat to squeak of make a strong aversive movement. This value was determined by the "up-down" method ever 5 minutes. Three stable consecutive 5 minute readings were obtained (a, b, and c) followed by an ip injection of drug, drug combination or saline vehicle in an observer blinded fashion. ECT readings were continued every 5 minutes for a further 30 minutes (readings d,e,f,g,h,i). Individual values of ECT measured in mA were standardised to minimise differences between rats due to electrode placement and resistance. This was achieved by dividing all individual readings taken by the mean of the first three pre-drug treatment readings (mean of a+b+c). All values, so transformed, were combined for testing time and drug treatment to calculate means ± SEM and plotted as time response curves shown in figure 2 for groups of rats that received the following treatments:

- Flupirtine at a dose of 5 mg/kg ip alone
- Flupirtine at a dose of 10 mg/kg ip alone
- Morphine at a dose of 0.4 mg/kg ip alone
- A combination of morphine at a dose of 0.4 mg/kg with flupirtine at a dose of 5 mg/kg

It can be seen from the curves shown in figure 2 that drug effects came on and reached a plateau 10-30 minutes after ip injection given after the reading taken at time 0. For statistical comparison all the values for rats in a group were combined for pre-treatment (all a,b and c values) and post-treatment (all e,f,g,h and i values). These are shown in table 3.

Table 3

SUMMARY DATA ECT PARADIGM		n rats	n observations	mean	SD
saline controls	pre	16	48	1.00	0.05
	post		90	1.27	0.35
flupirtine 5mg/kg	pre	20	60	1.00	0.05
	post		100	1.54	0.64
flupirtine 10mg/kg	pre	4	12	1.00	0.07
	post		20	1.92	0.79
morphine 0.4mg/kg	pre	12	36	1.00	0.06
	post		60	1.46	0.53
combination morphine 0.4 mg/kg and flupirtine 5mg/kg	pre	12	36	1.00	0.09
	post		60	1.91	0.89

A one way ANOVA with Tukey-Kramer post hoc test was applied to the data in the table above. ECT values after flupirtine 5 or 10 mg/kg, morphine 0.4 mg/kg or the combination of morphine 0.4 mg/kg with flupirtine 5 mg/kg were all significantly greater than saline ($p<0.001$). There was significant antinociception following flupirtine alone at 5 or 10 mg/kg and morphine 0.4 mg/kg ($p<0.001$). The amount of antinociception following morphine 0.4mg/kg/flupirtine 5mg/kg combination was significantly greater than morphine 0.4 mg/kg or flupirtine 5 mg/kg given alone ($p<0.001$). It is therefore concluded that non-sedative doses of flupirtine can increase the antinociception following morphine without causing sedation.

Example 4

Streptozotocin-induced Diabetic Neuropathy

The treatment of neuropathic pain states, including diabetic neuropathy in humans is frequently unsatisfactory. Current pharmacological regimens consist of the tricyclic antidepressants[10, 11, 12], anticonvulsants, systemic local anaesthetics (lignocaine) and mexiletine and, more recently, GABApentin. All have limited success[13, 14, 15, 16]. It is accepted generally that human neuropathic pain states are resistant to opioid treatment[13]. Some researchers have found that opioids may produce antinociceptive effects in neuropathic pain models but at higher than normal doses that also cause sedation revealed by tests such as open field activity monitoring and the rotarod test. This indicates a shift of the dose-response curve to the right, beyond the normal therapeutic range[17].

Courteix and co-workers have developed a diabetes-induced model for neuropathic pain. They found that induction of experimental insulin-dependent diabetes mellitus in rats caused allodynia and hyperalgesia[18]. They went on to show that intravenous morphine induced a dose-dependent antinociceptive effect at doses twice as high as those in normal rats, using the mechanical nociceptive paw pressure test[18]. Thus the diabetic model reproduced the experience of diabetic neuropathic pain in humans; it is opioid resistant. The experiments reported here use this model to assess the relative efficacy of flupirtine and morphine given alone and in combinations in causing antinociception assessed with paw pressure measured using the Randall Sellito method.

Male Wistar rats (wt 65 - 80g) were used for these experiments. Animals were housed 5 per cage under standard laboratory conditions. Food and water were provided ad libitum. In all the experiments attention was paid to ethical guidelines for the investigation of experimental pain in conscious animals[19]. All work was carried out with the permission from the Monash University Standing Committee On Ethics in Animal Experimentation (SCAE NUMBER 96-021).

Induction of diabetes / hyperalgesia

Rats were injected intraperitoneally (IP) with streptozotocin (STZ) (150 mg/kg total dose) (Sapphire Bioscience) dissolved in sodium chloride (0.9%). The 150mg dose was given in two 75mg/kg injections on consecutive days. Diabetes was confirmed one week after injection of STZ by measurement of tail vein blood glucose levels with Ames Glucofilm test strips and a reflectance colorimeter (Ames Glucometer 3, Bayer Diagnostics). Only animals with final blood glucose levels ≥15mM were deemed to be diabetic. The rats were retested for hyperglycaemia once per week to confirm continued high blood glucose readings. Hyperalgesia was assessed using the paw pressure test, previously described by Randall and Selitto[20].

Tests took place 5 weeks after the first injection of STZ. Animals that had paw pressure nociceptive thresholds below 30g (60 % of the value in normal weight matched rats) were

deemed to have developed hyperalgesia/neuropathic pain and thus used in further experiments.

Nociceptive tests

After the successful documentation of the development of hyperalgesia in diabetic animals by the paw pressure test, more extensive nociceptive testing paradigms were carried out in diabetic neuropathic animals and weight-matched controls; the control rats were 1 – 2 weeks younger. Paw pressure (PP) was measured by the method described by Randall and Selitto using a Ugo-Basile Algesimeter (Apelex; probe 1mm; weight: 10g)[20]; increasing pressure to the left hind paw was applied until vocalization was elicited. Paw withdrawal thresholds were measured in groups of rats 20 minutes and 10 minutes before, immediately before (time 0) and also at 20, 30 and 40 minutes after intraperitoneal (ip) injections of:

- saline (controls)
- weight matched non diabetic controls (no treatment)
- flupirtine 5mg/kg alone
- flupirtine 10 mg/kg alone
- morphine 1.6 mg/kg alone
- morphine 3.2 mg/kg alone
- flupirtine 5mg/kg plus morphine 3.2 mg/kg together
- flupirtine 10 mg/kg plus morphine 1.6 mg/kg together

Values in individuals were combined for each testing time to calculate means and SEM which were plotted on time response curves as shown in figure 3.

It can be seen that the values of the paw withdrawal thresholds measured at -20, -10 and at 0 were the same for all groups of diabetic rats and these values were significantly below those for normal weight matched controls; diabetes caused hyperalgesia. It can also be seen that the responses to drugs, if present were apparent at 20 minutes after the injection of drug or drug combination and the response was constant and stable between 20 to 40 minutes after the injection which was given at time 0. For each treatment group all the paw withdrawal threshold values taken at time -20, -10 and 0 (pre-drug) were combined as

were those taken at time +20, +30, and +40 (post-drug). Means and SD's were calculated for each group for pre- and post- drug administration, as shown in table 4 below. A one-way ANOVA was applied to the values in this table to compare the post drug values with the values for paw withdrawal thresholds in weight-matched non diabetic rats; a return of NS, no significant difference indicates that the drug or drug combination had reversed completely the diabetes-induced hyperalgesia. In addition a one way ANOVA was applied to the data in Table 4 to assess whether any of the drug treatments led to any antinociception i.e., was there a significant increase in paw withdrawal thresholds after the drug treatment compared with the paw withdrawal thresholds before the treatment.

Table 4

SUMMARY DATA – DIABETIC NEUROPATHY	n obs	mean	SD	n obs	mean	SD
weight matched non diabetic controls n = 21 rats	63	44.7	6.9			
saline controls n = 16 rats	48	28.54	4.12	48	30.94	5.89
flupirtine 5mg/kg alone n = 21 rats	63	28.25	4.50	63	31.90	7.15
flupirtine 10mg/kg alone n = 15 rats	45	27.89	5.69	45	41.00	14.56
morphine 1.6mg/kg alone n = 14 rats	42	28.10	5.84	42	31.90	6.98
morphine 3.2mg/kg alone n = 8 rats	24	26.67	4.82	24	35.00	10.11
flupirtine 5mg/kg + morphine 3.2mg/kg together n = 8 rats	24	26.67	4.08	24	36.88	12.84
flupirtine 10mg/kg + morphine 1.6mg/kg together n = 17 rats	51	28.82	5.16	51	49.41	15.55

Complete reversal of streptozotocin-induced diabetic hyperalgesia was caused by flupirtine 10 mg/kg given alone and also flupirtine 10mg/kg + morphine 1.6mg/kg together ($p>0.05$); i.e., the paw withdrawal thresholds after the drug treatment were not statistically different from thresholds for normal non-diabetic weight matched controls. Flupirtine 5mg/kg alone and morphine 1.6mg/kg alone cause no significant reversal of diabetes-induced hyperalgesia; the paw withdrawal thresholds after the drug injection were not significantly different compared with the thresholds in those rats measured before the drug was injected ($p>0.05$). Morphine 3.2 mg/kg given alone caused significant antinociception; paw thresholds did increase significantly after the drug ($p<0.05$) but those values and the size of that response were significantly less than that caused by a lower dose of morphine

(1.6mg/kg shown to be ineffective when it was given alone) given in combination with flupirtine 10mg/kg ($p < 0.001$). Finally, flupirtine 10mg/kg in combination with morphine 1.6mg/kg caused greater antinociception than flupirtine 10mg/kg alone.

Experimental Conclusion

We can conclude from the results reported in examples 2 to 4 that non-sedative doses of flupirtine can increase the overall antinociceptive effect of morphine without causing sedation in three animal models of pain; electrical, inflammatory and neuropathic. In neuropathic and inflammatory pain models it is possible, using flupirtine in combination with morphine, to cause such significant antinociception as to reverse hyperalgesia such that animals with these pain states are rendered normal with respect to pain sensitivity. This demonstrates utility of flupirtine as an adjunct to opioid analgesics especially in pain states such as inflammatory and neuropathic pain, which are either opioid resistant to the extent that only partial analgesia can be achieved with opioid drugs or are at doses that cause side effects such as sedation. The co-administration of flupirtine with the opioid offers improved pain control in inflammatory and neuropathic pain with doses and combinations that are not accompanied by sedation.

It should be understood that the present invention has been described by way of example only and that modifications and/or alterations thereto which would be apparent to a skilled person based upon the disclosure herein are also considered to fall within the scope and spirit of the invention.

REFERENCES

1. Merskey H, Bogduk N. Classification of Chronic Pain, 2nd edn. Seattle: IASP Press, 1994; 394

2. Woolf CJ and Mannion RJ. Pain: neuropathic pain: aetiology, symptoms, mechanisms and management. Lancet 1999; **353**: 1959–64

3. McQuay HJ, Tramer M, Nye BA, Carroll D, Wiffen PJ, Moore RA. A systematic review of antidepressants in neuropathic pain. Pain 1996; **68**: 217–27

4. Sindrup SH, Jensen TS. Efficacy of pharmacological treatments of neuropathic pain: an update and effect related to mechanism of drug action. *Pain* 1999; **83**: 389–400

5. Rowbotham MC. Kalso E, McQuay HJ, Wiesenfeld-Hallin Z, eds. The debate over opioids and neuropathic pain. Opioid Sensitivity of Chronic Non-cancer Pain. Seattle: IASP Press, 1999; **18**: 307–17

6. Nickel, H.O., Barbe, I., Szelenyi, Arzneim. – Forsch. 40(II)8, 905-908 (1990)

7. Nickel, B., Herz, A., Jakovlev, V., Tibes, U., Arzneim. – Forsch. 35(II), 1402 (1985)

8. Bundgaard, H., Design of Prodrugs, Elsevier, 1985

9. Remmingtons Pharmaceutical Sciences 18th Edition, 1990, Mack Publishing Co., Easton, Pennsylvania, USA

10. Sindrup, S.H., Gram, L.F., Brosen, K., Eshoj, O. and Mogensen, E.F., The selective serotonin reuptake inhibitor paroxetine is effective in the treatment of diabetic neuropathy symptoms, Pain, 42 (1990) 135-144.

11. Max, M.B., Towards physiologically based treatment of patients with neuropathic pain, Pain, 42 (1990) 131-133.

12. Max, M.B., U.S. government disseminates acute pain treatment guidelines: Will they make a difference, Pain, 50 (1992) 3-4.

13. Arner, S. and Meyerson, B.A., Lack of analgesic effect of opioids on neuropathic and idiopathic forms of pain, Pain, 33 (1988) 11-23.

14. Davis, B.L., Manzanares, J., Lookingland, K.J., Moore, K.E. and Clemens, L.G., Noradrenergic innervation to the VMN or MPN is not necessary for lordosis, Pharmacology, Biochemistry and Behavior, 39 (1991) 737-742.

15. Galer, B.S., Neuropathic pain of peripheral origin: Advances in pharmacologic treatment, Neurology, 45 Suppl. 9 (1995) S17-S25

16. Avidan, A., Devor, M., Shir, Cotev, S., Eimerl, Krochek and Peruansky, 1995 intravenous lidocaine infusion for chronic pain therapy, Israel Journal of Medical Sciences, 32 (1996) 331-334.

17. Portenoy RK, Foley KM, Inturrisi CE. The nature of opioid responsiveness and its implications for neuropathic pain: new hypotheses derived from studies of opioid infusions. Pain. 1990 Dec;43(3):273-86.

18. Courteix, C., Eschalier, A. and Lavarenne, J., Streptozocin-induced diabetic rats: behavioural evidence for a model of chronic pain, Pain, 53 (1993) 81-88.

19. Zimmerman, M., Ethical guidelines for investigation of experimental pain on conscious animals, Pain, 16 (1983) 109-110.

20. Randall, L.O., and Selitto, J.J., A method for measurement of analgesic activity of inflamed tissue, Archiv. Inst. Pharmacdynamie, 1957, 111, 409.

DATED this 16th day of December, 2003

Monash University

By DAVIES COLLISON CAVE

Patent Attorneys for the Applicant

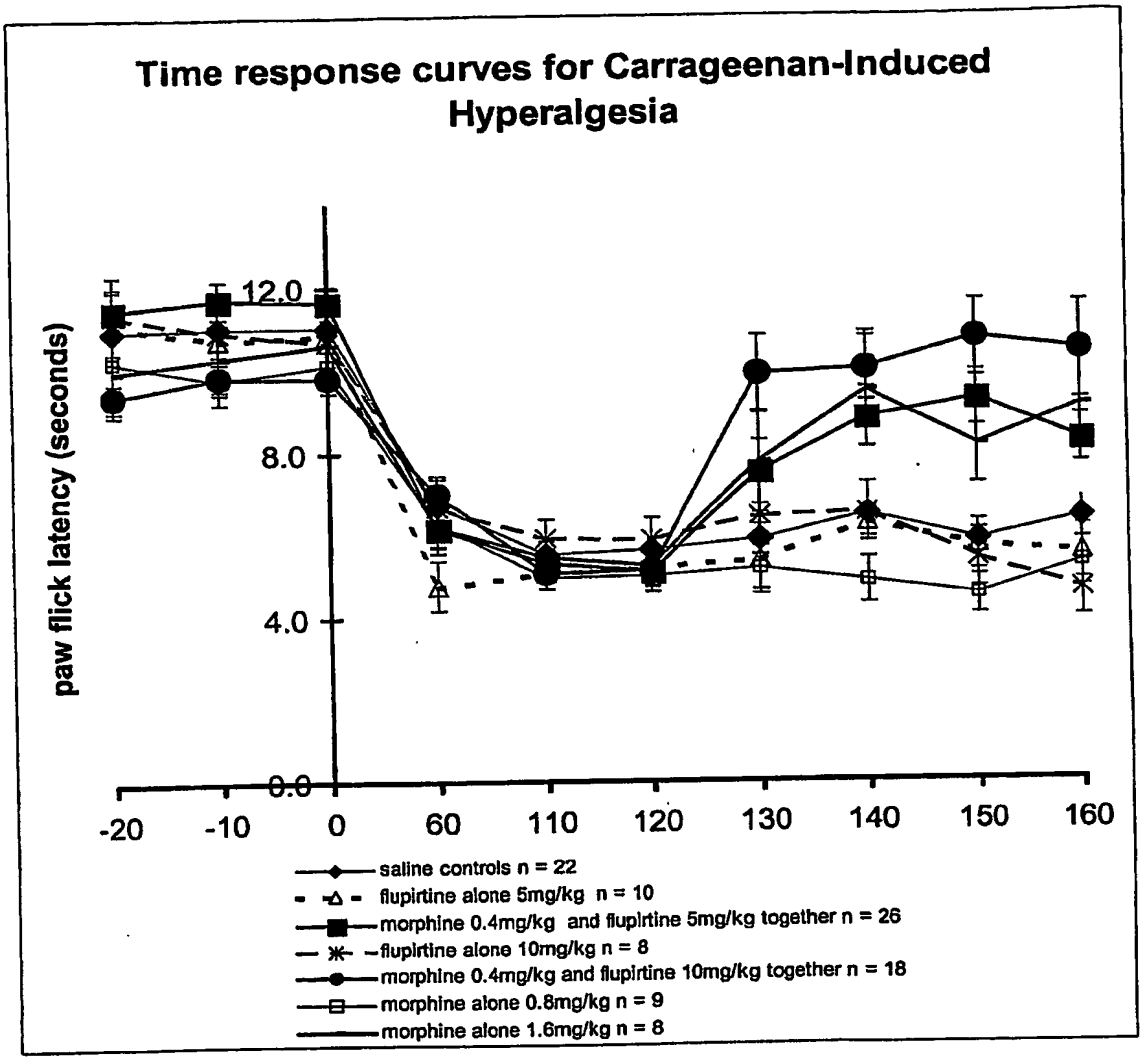

Time response curves for Carrageenan-Induced Hyperalgesia
paw flick latency (seconds)
12.0
8.0
4.0
0.0
-20
-10
0
60
110
120
130
140
150
160
saline controls n = 22
flupirtine alone 5mg/kg n = 10
morphine 0.4mg/kg and flupirtine 5mg/kg together n = 26
flupirtine alone 10mg/kg n = 8
morphine 0.4mg/kg and flupirtine 10mg/kg together n = 18
morphine alone 0.8mg/kg n = 9
morphine alone 1.6mg/kg n = 8

FIGURE 1

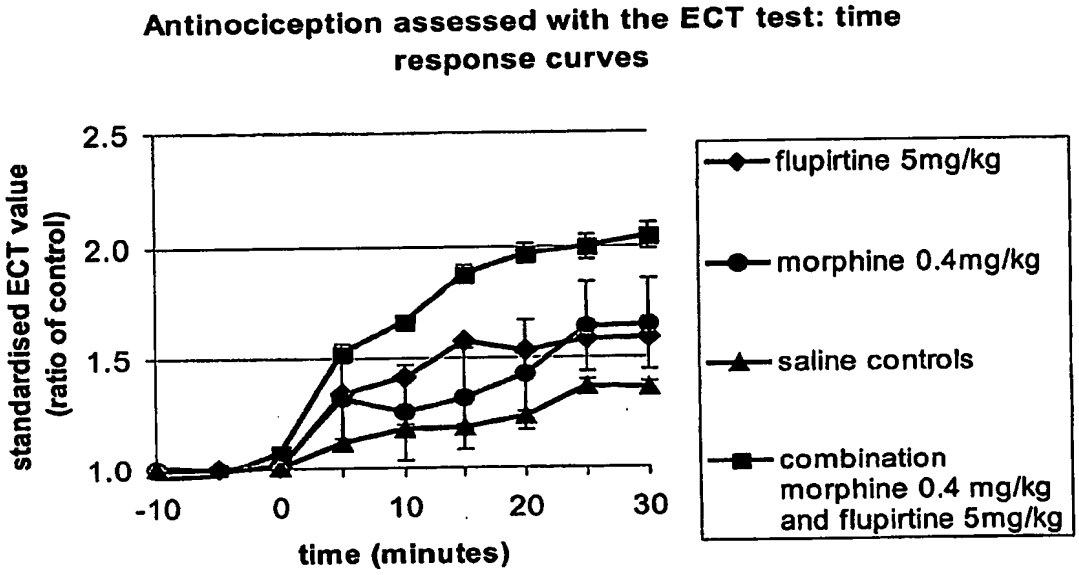

Antinociception assessed with the ECT test: time response curves
standardised ECT value (ratio of control)
2.5
2.0
1.5
1.0
-10
0
10
20
30
time (minutes)
flupirtine 5mg/kg
morphine 0.4mg/kg
saline controls
combination morphine 0.4 mg/kg and flupirtine 5mg/kg

FIGURE 2

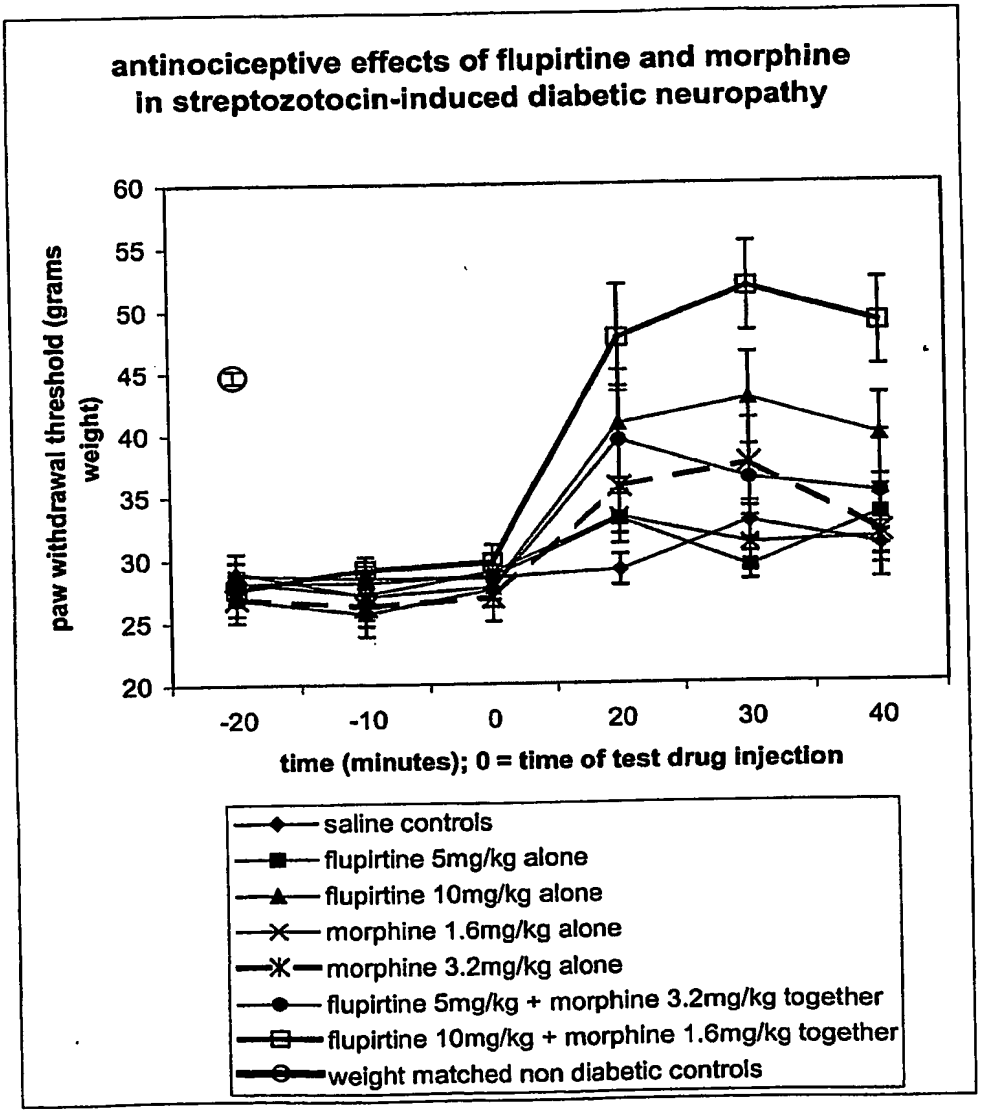

antinociceptive effects of flupirtine and morphine in streptozotocin-induced diabetic neuropathy
paw withdrawal threshold (grams weight)
60
55
50
45
40
35
30
25
20
-20
-10
0
20
30
40
time (minutes); 0 = time of test drug injection
saline controls
flupirtine 5mg/kg alone
flupirtine 10mg/kg alone
morphine 1.6mg/kg alone
morphine 3.2mg/kg alone
flupirtine 5mg/kg + morphine 3.2mg/kg together
flupirtine 10mg/kg + morphine 1.6mg/kg together
weight matched non diabetic controls

FIGURE 3

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/AU04/001772

International filing date: 16 December 2004 (16.12.2004)

Document type: Certified copy of priority document

Document details: Country/Office: AU
Number: 2003906981
Filing date: 16 December 2003 (16.12.2003)

Date of receipt at the International Bureau: 17 January 2005 (17.01.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)